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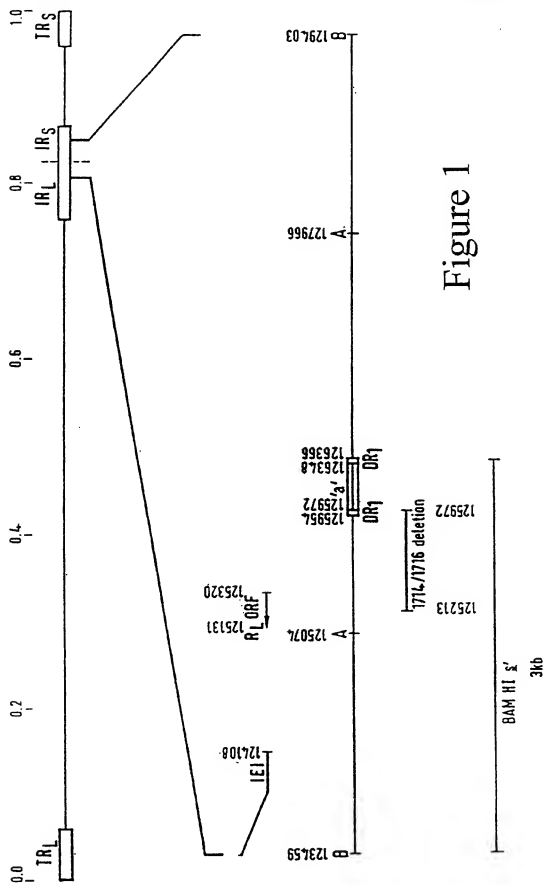
(19) **United States**(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0271620 A1**
Brown et al. (43) **Pub. Date: Dec. 8, 2005**(54) **HERPES SIMPLEX VIRUS COMPLEX**(30) **Foreign Application Priority Data**(76) Inventors: **Susanne Moira Brown, Glasgow (GB);**
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C12N 15/86(52) **U.S. Cl.** **424/93.2; 435/235.1; 435/456**(57) **ABSTRACT**

There is provided an HSV complex which comprises an avirulent HSV and a targeting agent which allows the HSV particle to infect and lyse a specific targeted cell. The inventors have found a way in which avirulent HSV can be targeted to disease cells, e.g. cancer cells, by incorporating an antibody binding domain into one or more viral glycoproteins.

(21) Appl. No.: **10/504,460**(22) PCT Filed: **Feb. 12, 2003**(86) PCT No.: **PCT/GB03/00603**



a) GAATTCATGGATGGAGCTGTATCATCCTCTTCTTGGTAGCACAGCTACAGGTGTCCACTCCGCGGCCACGCCGCCGGATGTGCAACTGGTGGAGTCT

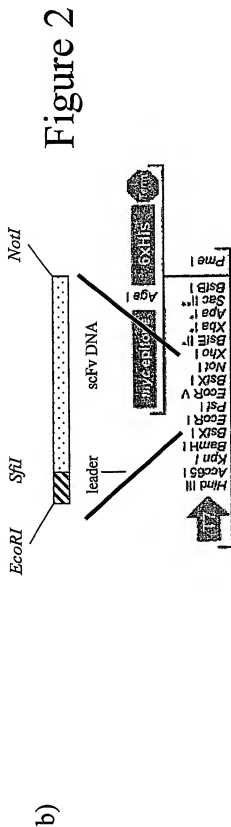


Figure 2

*There is a unique BstE II site, but no Xba I or Apa I sites in version C.

**There is a unique Sac II site between the Age I site and the BstE II site in version B only.

Comments for pcDNA4/myc-His[®]
5075 nucleotides

Figure 3

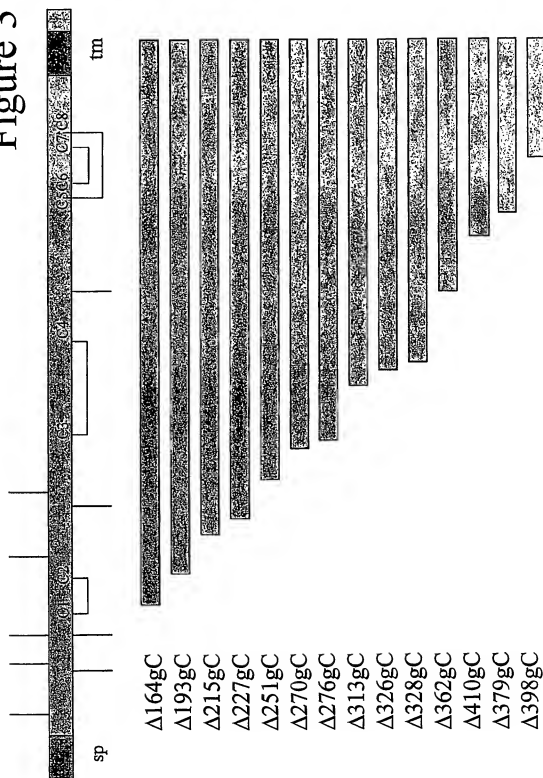


Figure 4

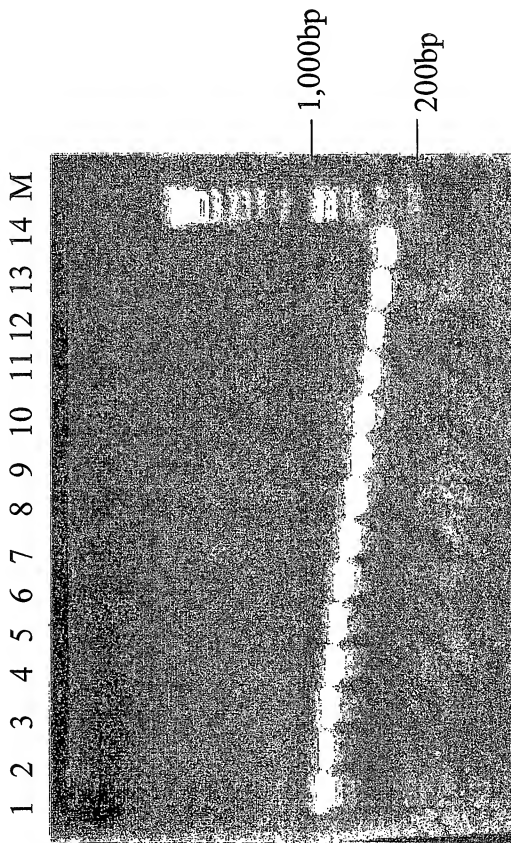
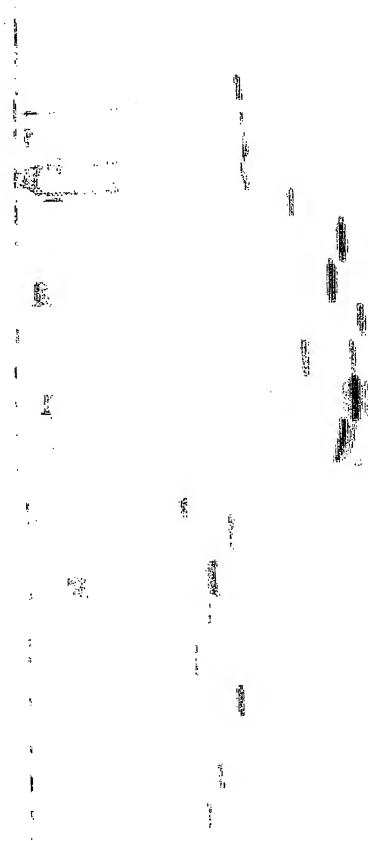


Figure 5

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20



HERPES SIMPLEX VIRUS COMPLEX

[0001] The present invention relates to Herpes Simplex Virus (HSV) complex, including its production and its use. Particularly, but not exclusively, the invention relates to an HSV type I incorporating an antibody binding domain for targeting cells especially cancer cells.

[0002] HSV is an enveloped, icosahedral, double-stranded DNA virus that infects mammals, including humans. Wild-type HSV infects and replicates in both terminally differentiated cells and dividing cells. Wild type HSV is neurovirulent, entering the peripheral nervous system where active viral replication is suppressed and the virus remains latent in neurons. HSV can reactivate from the latent state to produce infectious lesions. The HSV neurovirulence gene ICP34.5 is believed to condition post-mitotic cells (particularly neurons) for viral replication. (Thompson et al. *Virology* 172, 435-450 (1989); MacLean et al., *J. Gen. Virol.* 72, 631-639 (1991); Harland and Brown, *J. Gen. Virol.* 66, 1305-1321 (1985); and Taha et al., *J. Gen. Virol.* 70, 705-716 (1989)). ICP34.5 deletion mutants cannot replicate in terminally differentiated cells but can lytically infect dividing cells (Brown, S. M. et al. 75, 2367-2377 (1994)). The HSV-1 mutant strain 1716 is an ICP34.5 deletion mutant (EP-B-0 571,410) of HSV type I strain 17, that has reduced virulence and greatly reduced lethality in mice, but it replicates as efficiently as wild type virus in actively dividing tissue culture cells (Maclean, A. R. et al. *J. Gen. Virol.* 72 631-639 (1991), Brown, S. M. et al., *J. Gen. Virol.* 75 2367-2377 (1994)). The ability of ICP34.5 deletion mutants to specifically target and lyse dividing cells and not post mitotic cells makes them an attractive therapeutic agent for the treatment of cancer. HSV infection of rapidly dividing cancer cells leads to death of the cells by lysis. The 1716 mutant kills tumour cell lines in tissue culture and, in a range of in vivo cancer models, the virus was shown to induce tumour regression and prolong survival (Kesari, S., Randazzo, B. P. and Valyi-Nagy, T. *Lab Invest.* 73 636-648 (1995), MacKie E. A. et al. *Br. J. Cancer* 74 745-752 (1996), Randazzo, B. P. et al. *J. Invest Dermatol.* 108 933-937 (1997)). In clinical trials, direct injection of 1716 was effective in treating patients with recurrent glioma (Rampling, R. et al. *Gene Therapy* 7 (10) 859-866 (2000)) and metastatic melanoma (MacKie, R. M., Stewart, B. and Brown, S. M. *The Lancet* 357 525-526 (2001)).

[0003] Significantly, in both of these trials there was no evidence for spread of 1716 to surrounding normal tissue. A second HSV-1 ICP34.5 deletion mutant, G207, which additionally lacks the UL39 gene that encodes the large subunit of the viral ribonucleotide reductase, has also been shown to be safe and effective in patients with malignant glioma (Markert, J. M. et al. *Gene Therapy* 7 (10), 867-874 (2000)).

[0004] Although strains such as 1716 and G207 that have impaired neurovirulence and that selectively infect dividing cells have strong therapeutic potential for the treatment of human malignancies, some limitations of their use are anticipated by the inventors. 1716 is able to infect and kill a variety of tumour cells in tissue culture, but its permissive range in vivo may be more restricted. For example, 1716 infection of B or T cell lymphomas has not been reported. Additionally, the virus may infect tumour cells less efficiently in vivo than in cell culture. The inventors have appreciated that it is desirable to overcome certain cell-type

restrictions and increase the efficiency of infection of tumour cells so that modified HSV can be more widely, effectively and safely applied as an in vivo therapy.

[0005] Broadly, the present invention provides a means of altering or modifying the tropism of HSV, so that a particular range of cell types can be targeted.

[0006] At its most general, the present invention provides an HSV complex comprising a modified HSV and a targeting agent capable of targeting said modified HSV to a specific cell type, preferably a proliferating cell e.g. a cancer cell. The invention further provides a method of producing the HSV complex and its use of the complex in the treatment of diseases such as cancer.

[0007] Thus, in a first aspect, the present invention provides an HSV complex comprising an HSV linked to a targeting agent, capable of targeting a specific cell type, where the genome of said HSV is modified in the terminal portion of R_L within Bam HI 1 s (0.0-0.2 and 0.81-0.83 mu).

[0008] The targeting agent is conveniently an antibody or component of an antibody, e.g. an antibody binding domain. The antibody is preferably capable of specifically binding to a cell surface protein present of the cell type targeted. This is discussed below. The targeting agent is preferably linked to the modified virus via a viral envelope protein so that it is displayed on the surface of the virus. A convenient way of achieving this is to form a fusion protein comprising the targeting agent and a viral envelope protein such as a glycoprotein. Alternatively, the targeting agent may be linked to the viral particle by chemical means, e.g. covalently, or by a binding agent, e.g. avidin/streptavidin and biotin.

[0009] In a preferred embodiment, nucleic acid encoding the targeting agent is incorporated into the viral genome so that it is expressed as a fusion protein with a viral envelope protein e.g. a glycoprotein, and as a result displayed on the surface of the particle.

[0010] Thus, the invention provides an HSV which is capable of targeting a specific cell type, said HSV lacking an expressible $\gamma 34.5$ gene so as to lack neurovirulence and wherein the HSV expresses a targeting agent.

[0011] As an antibody binding domain forms a preferred embodiment of the invention, the following description will concentrate on the use of antibodies. However, it will be apparent to the skilled person that other targeting agents may be used, e.g. members of a specific binding pair such as a receptor and its ligand.

[0012] The antibody or antibody component incorporated into the viral envelope influences the selectivity of the virus by enhancing the efficiency of viral infection of a certain cell type or cell types. The HSV infection process is initiated through contact between glycoproteins of the viral envelope and glycoproteins of the target cell membrane. In the present invention, antibodies with specific affinity for membrane proteins of the chosen cell type are incorporated into the HSV viral envelope, increasing the affinity of the HSV for the surface of the chosen target cell through the additional interaction between the antibody and the cell surface protein. The binding of the antibody binding domain to its target antigen on the cell surface will bring both virion and cellular membranes into closer proximity and allow the viral enve-

lope glycoproteins to initiate fusion of the membranes, leading to penetration of the cell.

[0013] The HSV-1 virion envelope contains at least 10 integral membrane glycoproteins, several of which mediate entry into mammalian cells. The initial interactions between the virus and the cell are between viral glycoproteins gB and/or gC and cell surface heparan sulphate proteoglycans, but this interaction is insufficient for viral penetration. Fusion of the viral and cellular membranes requires gD, gB and a gH/gL complex, and these proteins are presumed to act in concert. Specific receptor-mediated entry of HSV-1 involves interaction of gD with HVEM/HveA (herpesvirus entry mediator A), a lymphotoxin receptor and member of the TNF receptor family. Expression of HveA in CHO cells that are normally refractory to viral penetration rendered them permissive. Several other receptors have been identified using non-permissive CHO cells including the poliovirus receptor related proteins 1 and 2, now renamed HveC and HveCB respectively. For this work, the inventors have been able to render a cell line normally resistant to infection permissive for HSV-1 entry.

[0014] The present invention uses an HSV that has an impaired ability to infect, replicate in or lyse terminally differentiated, non-dividing cells. In this form the virus is particularly suited for use as a therapeutic agent to treat diseases associated with proliferating cells such as cancer and non-cancer diseases such as Crohn's disease. The inventors believe the modified virus in accordance with the present invention will be particularly useful in targeting proliferating T-cells in cancer or non-cancer situations.

[0015] In a preferred embodiment, the HSV has been modified so that the gene encoding ICP 34.5 (gene γ 34.5) is incapable of expressing a functional gene product.

[0016] The modified virus preferably contains a modification in respect of the wild type virus within the Bam HI s region of the internal repeat R_L (0.81-0.83 mu) and within the counterpart region of the terminal R_L (0-0.02 mu) such that the modified virus (variant) lacks neurovirulence.

[0017] The modification of the virus genome may be achieved by deletion of one or more nucleotides, insertion of additional nucleotides or any other alteration of the nucleotide sequence such as rearrangement, or substitution. Preferably, the modification of the HSV genome is achieved by deletion of one or more nucleotides.

[0018] The HSV may be a spontaneously isolated deletion variant of the wild type or it may be a wild type strain into which the desired modification has been introduced. Such modifications in the HSV may be made by genetic manipulation, for example by site-directed mutagenesis, or by excision of a portion of the genome with or without replacement with a pre-prepared DNA cassette incorporating the required modification.

[0019] Preferably, the HSV is HSV type 1 (HSV-1) even more preferably HSV-1 strain 17. In one embodiment, the HSV-1 strain will have a deletion of at least 100 nucleotides in the Bam HI s' region between Alu I site at 125074 nb and 125972 nb and within its counterpart sequence in TR_L .

[0020] More preferably 0.5 to 3 kb of the Bam HI s' region and its counterpart in TR_L is deleted. Still more preferably about 0.7-2.5 kb is deleted.

[0021] Suitable modified HSV include HSV-1 mutant 1716 or G207. The production of HSV1716 is described in EP 571,410-B which is incorporated herein by reference.

[0022] In addition to the above, the inventors have appreciated that, in order to treat a diverse range of tumours, the HSV complex in accordance with the invention will ideally have to be administered into the circulation of a patient. However, not only does the virus have to find the tumour cells (it can bind and be adsorbed by many different cell types) but it also has to contend with pre-existing immunological defences (e.g. neutralising antibodies) designed to eliminate the virus. Pre-existing immunological defences will be reasonably common as most people have had previous exposure to HSV-1. Given this, the present inventors have appreciated that there is a need to develop a "stealth" virus that avoids immunological detection and can specifically target tumour cells. Accordingly, the inventors have produced a stealth virus by eliminating the normal viral glycoproteins that mediate cell adsorption and replacing them with antibody-directed entry mediating glycoproteins incorporated into the virion structure. The principal viral glycoproteins involved in cell entry also provide the main neutralising epitopes and their removal will minimise immunological activity against such a virus. Thus, tumour antigen-directed HSV e.g. HSV1716 introduced into the circulation can target many tumour types including disseminated cancers that are either inaccessible or too numerous for direct injection or are too small to be detected.

[0023] Thus, the HSV complex as described above, e.g. HSV1716, that displays a tumour specific targeting antibody in accordance with the first aspect of the present invention, may be modified such that the genes encoding viral glycoproteins essential for normal cell entry (e.g. principally gD but also gC and/or gB, see below) are deleted or inactivated, thereby rendering the resulting virus dependent on tumour antigen/antibody interactions as the main route for cell infection. Deletion of these glycoproteins from the virus particle also removes the principal neutralising epitopes and therefore greatly reduces immunological defences when administered systemically.

[0024] Therefore, the HSV complex according to the first aspect of the present invention may be further modified so that one or more viral glycoproteins, e.g. gD, gC and/or gB, are inactivated or deleted such that they are unable to mediate entry of the viral particle into cells. It is preferable that the one or more glycoproteins are modified at the genome level such that they cannot be expressed or cannot be expressed in a functional form. It is most preferable that the HSV genome is modified so that the one or more glycoproteins cannot be expressed at all as this will provide the HSV variant with the additional advantage that it can escape any pre-existing immunological defences in vivo.

[0025] As mentioned above, the various glycoproteins may be modified or deleted from the viral particle, preferably at a nucleic acid level. The modification may include the incorporation of nucleic acid encoding the targeting agent so that the targeting agent is expressed on the surface of the particle. Thus, the HSV genome may be modified, in addition to the γ 34.5 gene, such that one or more of the glycoproteins (e.g. gD, gC and/or gB) express the targeting agent, e.g. the antibody binding domain.

[0026] Preferably, the antibody or antibody component is specific for tumour surface antigen, i.e. antigen found on the

surface of a tumour cell and associated with that cell, being either unique to tumour cells or being more abundant on tumour cells than on most if not all non-tumour cells. Many novel or a typical forms of normal proteins are expressed by tumour cells, and antibodies directed against these provide tumour targeting strategies. For example, carcinoembryonic antigen (CEA) is an important marker on many tumour cells and engineered antibodies directed against CEA have undergone clinical trials (Mayer, A. et al., *J. Immunol. Methods* 231 261-273 (1999)). Engineered antibodies directed against the Her2/neu growth factor (Trastuzumab) and against CD20 (rituximab) have been licensed for the treatment of breast cancer and Non-Hodgkin's lymphoma respectively Holliger, P. and Hoogenboom, H. (1998), *Nature Biotechnology* 16, 1015. CD55 (decay accelerating factor) is over-expressed by tumour cells to block complement activation and antibodies directed against CD55 may have therapeutic potential (Li, L. et al. B. J. *Cancer* 84 (1) 80-86 (2001)). Incorporation of an antibody binding domain that specifically targets tumour antigens such as CEA, Her2, CD20 and CD55 into the envelope of HSV will have the potential to alter its cellular tropism thus allowing infection of non-permissive tumour cells and possibly improving its ability to infect other tumour cells. For example, 1716 virions that display an antibody binding domain specific for CD20 may be able to infect and kill B-cell lymphomas.

[0027] Further, HSV, e.g. HSV1716 virions that display tumour targeting antibodies and from which the normal HSV-1 entry glycoproteins are deleted will only infect the targeted tumour cells.

[0028] The antibody binding domain may have specific affinity for a cell surface protein found on the cell type from which the tumour originated, e.g. in the case of a glioma, the antibody or antibody component incorporated into the HSV viral envelope would be specific for an antigen commonly associated with glial cells. The specificity of the avirulent HSV strain for infecting dividing cells would therefore be further modified so that glial cells were preferentially infected by the virus more than other types of dividing cells. By targeting dividing glial cells, the HSV should infect and lyse glioma cells more efficiently than any other cells. The use of antibodies or antibody components against particular cell types can also be used to extend the tropism of HSV to cell types that are not otherwise efficiently infected by HSV, e.g. the use of antibodies or antibody components specific for antigen found on B cells would be expected to extend the tropism of HSV to B cells. Antibodies or antibody components of different specificities may be included together in one HSV viral envelope. The combination of these specificities would be expected to give greater specificity of targeting to the desired cell type.

[0029] The antibody binding domain would preferably be fused to an integral membrane protein in the viral envelope, preferably an HSV glycoprotein. The preferred HSV-1 and HSV-2 glycoproteins are gB, gC and gD.

[0030] In a preferred embodiment, the antibody binding domain is in the form of a single chain variable fragment (scFv).

[0031] In a second aspect, the present invention comprises a method of making a modified HSV complex according to the first aspect of the invention comprising the step of

infecting a cell line that constitutively expresses said fusion protein with an HSV, preferably a modified HSV, more preferably modified HSV-1.

[0032] In a third aspect, the present invention comprises a method of making a modified HSV complex according to the first aspect of the invention comprising the step of incorporating DNA encoding said fusion protein into the viral genome of the modified HSV.

[0033] In a fourth aspect, the present invention comprises the use of a modified HSV complex according to the first aspect of the invention in a method of medical treatment.

[0034] Preferably, the method comprises administering the HSV modified complex to a patient suffering from a disease associated with proliferation of cells, e.g. cancer, wherein the HSV complex selectively lyses dividing cells.

[0035] There is also provided a pharmaceutical composition which comprises the modified HSV in accordance with the present invention and a pharmaceutically acceptable carrier.

[0036] Pharmaceutical compositions comprising the modified HSV may be administered intravenously or directly injected into a tumour or infected site.

[0037] Aspects and embodiments of the present invention will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

[0038] FIG. 1 shows (a) the HSV-1 genome (with map units marked) in the prototype orientation; and (b) an expansion of BamHI k(s+q). The BamHI (B) and Alu (A) sites flanking the deletion in 1714/1716 are marked. All coordinates are based on the numbering of McGeoch et al. (1988). Also indicated are the positions of the 5' end of IE1, the "a" sequence, the DR₁/U_L boundary in the "a" sequence, a 189 bp conserved open reading frame between HSV-1 and HSV-2 (R₁ ORF) and the end points of the 759 bp deletion in 1714/1716. The deletion extends from the DR₁/U_L boundary to remove the 5' 107 bp of the R₁ ORF.

[0039] FIG. 2. Construction of the vector pEL4. a) Sequence of the 5' 99-mer oligonucleotide used to PCR amplify scFv DNA and simultaneously insert an IgG VH leader sequence. The EcoRI site is underlined in red and the SfiI site is underlined in purple. The sequence between these two sites encodes the IgG VH leader sequence that will target the expressed protein to the secretory pathway of the cell. The sequence downstream of the SfiI site is from IgG VH. The 3' primer was from IgG VL and inserts a NotI site allowing PCR amplification of scFv DNA with 5' and 3' SfiI and NotI sites respectively. Following PCR the scFv DNA with IgG VH leader was cloned into the plasmid pcDNA4A to give pEL4.

[0040] FIG. 3. Scale drawing of PCR-created N terminal deletions of HSV-1 gC. The full length gC protein is depicted at the top showing the signal peptide (sp) and transmembrane domain (tm). The 14 gC deletions shown below are designated according to the amino acid at their new N terminus. Thus A164gC has 163 amino acids deleted from its N terminus to give a gC fragment corresponding to amino acids 164-511.

[0041] FIG. 4. 1% agarose gel that shows PCR amplification of sequentially deleted gC DNAs (lanes 1-14). M=DNA ladder with the location of the 200 bp and 1000 bp bands indicated. A common 3' PCR primer, corresponding to the C-terminus of gC but without the stop codons inserts an XbaI site and allows cloning in-frame with the vector myc and 6-his tags. The 5' primers used were randomly selected from the gC coding sequence to give sequential deletions of between 6-90 nucleotides. The 5' primer inserts a NotI restriction site that allows the gC DNA to be cloned in-frame with the scFv DNA in pEL4. NotI/XbaI digested PCR amplified DNAs were cloned into pEL4 digested also with these enzymes to give scFv/gC fusion protein expression constructs.

[0042] FIG. 5. Western blots that show expression of scFv/gC fusion proteins in whole cell extracts and their incorporation into HSV1716 virus particles. Lanes 5-8, 9-11, 16 and 17 are whole cell extracts. Lanes 1-4, 12-15 and 18 are virus preparations. Lanes 1 and 5=Δ193gC, lanes 2 and 6=Δ215gC, lanes 3 and 7=Δ251gC, lanes 4 and 8=Δ328gC, lanes 9 and 13=Δ410gC, lanes 10 and 14=Δ424gC, lanes 11 and 15=Δ362gC, lane 12=Δ457gC, lanes 16-18=Δ227gC. Lanes 19 and 20 show mock virus preparations made from cell lines expressing Δ227gC and Δ424gC respectively, no bands were detected in these samples. Note that the cell line expressing Δ251gC gives a strong band in the cell extract (lane 7) but shows only a weak band in the virus preparation (lane 3). In contrast to this, Δ328gC is expressed at intermediate levels in the cell line but gives a strong band with its virus preparation. The overall results are summarized in Table 1.

EXAMPLE 1

Method of Making HSV-1 1716 Strains wherein Fusion Proteins Between Anti-Tumour scFvs and Envelope Glycoproteins B, C and D are Incorporated into the Virion Envelope

[0043] Recombinant scFv variants of monoclonal antibodies that bind different extracellular epitopes of CD55 are derived by standard protocols (see Pope, A. R., Embleton, M. J. and Mernaugh, R. (1996) In Antibody Engineering (eds McCafferty, Hoogenboom and Chiswell) Practical Approach Series, Oxford University Press Inc., New York. 1-40). scFv are particularly suitable for incorporation into the fusion protein because they are encoded by a single nucleotide sequence. scFv can be conveniently engineered using recombinant antibody technology (Hoogenboom, H. R. and Chames, P. Immunology Today 21 (8) 371-377 (2000)). Recombinant antibodies are predominantly produced using mRNA isolated from hybridomas or from populations of lymphocytes isolated either from the spleens of immunised animals or from human peripheral blood. DNA fragments encoding immunoglobulin heavy (V_H) and light (V_L) chain binding sites are amplified separately by RT-PCR and fused to produce fragments encoding single chain antibody molecules (the scFv). The scFv polypeptide effectively recreates the antigen recognition site in a single protein that retains high affinity binding. The cloned scFv can readily be genetically fused with the domains of other proteins, for example, with the M13 pIII coat protein for display on phages or to the enzyme carboxypeptidase G2 for antigen directed enzyme prodrug therapy (ADEPT).

[0044] Following RT-PCR cloning, sequencing and linkage of the antibody VH and VL for each monoclonal antibody, the scFv-encoding DNAs will be amplified using PCR primers that incorporate SfiI and NotI restriction enzyme sites at the 5' and 3' ends respectively for cloning in the phagemid vector pHEN2 or for construction of fusion proteins. The choice of restriction enzyme sites will depend on the sequences of the scFv and glycoproteins used. *E. coli* HB2151 will be transformed with the phagemid vectors and scFvs expressed by IPTG induction. ScFv expressed from pHEN2 have c-myc and 6-his tags for purification/detection. The reactivities of the recombinant scFv will be compared with their respective monoclonal counterparts by Western Blotting and IACS analysis using CHO cells stably transfected with plasmid that expresses their target antigens.

[0045] DNA encoding an scFv with an IgG VH leader sequence was cloned into the plasmid pcDNA4 (Invitrogen) to create the vector pEL4 (FIG. 2). The 5' primer used to amplify the scFv DNA incorporates an IgG VH leader sequence, linked to the scFv DNA by a SfiI site. The primer also inserts an EcoRI site 5' to the IgG VH leader sequence. The scFv DNA can be removed and replaced by alternative scFv DNAs using SfiI/NotI digestion. The leader sequence can be removed by EcoRI/SfiI digestion and replaced with other leader sequences, eg gC signal peptide sequences. Such leader sequences with EcoRI/SfiI restriction sites can be obtained either by PCR using appropriate primers or by using chemically synthesised complementary oligonucleotides.

[0046] A number of HSV-1 strain 17gB, gC and gD DNA fragments are PCR-cloned from the viral genome using methods which have previously been successful for other herpesvirus proteins (Sun, Y. and Conner, J. (1999) The U28 ORF of human herpesvirus-7 does not encode a functional ribonucleotide reductase R1 subunit. Journal of General Virology 80: 2713-2718. Sun, Y. and Conner, J. (2000) Characterisation of hetero-subunit complexes formed by herpes simplex type 1 and equine herpes virus type 4 ribonucleotide reductase R1 and R2 subunits. Biochem J. 347, No 1: 97-104. Incorporated herein by reference. The primers used to amplify the DNAs will incorporate appropriate restriction enzyme sites for fusion to the scFv DNA and cloning into pEL4. PCR primers for amplification of glycoprotein DNA will be designed such that a series of random, sequentially deleted N-terminally truncated proteins are expressed, each deletion will remove approximately 2-30 amino acids up to the region encoding the transmembrane domain. For example, gC comprises 511 amino acids with the transmembrane region located downstream of amino acid 479, a family of 14 sequentially deleted N-terminally truncated polypeptides (FIG. 3) for fusion to scFvs have been cloned. Examples of PCR-amplified gC DNAs are shown in FIG. 4. The primers used for PCR amplification of the gC DNA fragments incorporated NotI and XbaI sites at the 5' and 3' ends respectively. PCR-amplified DNA was digested directly with the appropriate enzymes (i.e. NotI/XbaI for gC fragments) and cloned into pEL4 digested also with these enzymes. The resulting constructs express scFv/gC fusion proteins with C-terminal myc and 6-his tags, under control of the CMV IE promoter. The promoter and tags are provided by the pcDNA4 backbone of pEL4 as is a zeocin resistance gene that allows production of stable cell lines using antibiotic selection. DNA fragments were also cloned into the PCR-cloning

vector pGEM-T Easy and sequenced to ensure that no PCR-induced mutations have been incorporated.

[0047] BHK cells were transiently transfected with each of the pEL4 constructs using lipofectamine and, after 48 hrs, zeocin selection (10 µg/ml) was initiated. Cells were selected with zeocin for approximately 21 days and extracts prepared for Western blotting. For each cell line, a polypeptide of the appropriate molecular size for the scFv/gC fusion protein was detected with the anti-myc tag monoclonal antibody 9B11 (New England Biolabs).

[0048] Examples of expression are shown in FIG. 5 and estimates for the levels of expression in each of the cell lines using the Western blot data are presented in Table 1. Immunofluorescence using 9B11 and an anti-murine IgG/FITC conjugate demonstrated a perinuclear/Golgi localisation for all of the expressed fusion proteins. Incorporation of scFv/glycoprotein fusion proteins into the HSV1716 envelope using stably transfected BHK expressing cell lines was analysed by Western blotting with 9B11. HSV1716 at 10 pfu/cell was used to infect each of the cell lines and virus harvested from the culture medium 24-28 hours later was analysed by Western blotting with 9B11. Examples are shown in FIG. 5 and results summarised in Table 1. No myc-tagged proteins were detected in similar preparations made from mock-infected cells. Most scFv/gC fusion proteins were incorporated into the virus (Table 1). Some were more efficiently incorporated than others (eg Δ328gC and Δ457gC are expressed at intermediate levels in their respective cell lines but are present as strong bands in their virus preparations) whereas incorporation of others expressed at high levels in their cell line was poor (eg Δ25IgC).

[0049] Recombinant viruses expressing the most appropriate scFv/glycoprotein fusions are created using a variant of 1716 that expresses green fluorescent protein (GFP). Incorporation of scFv/glycoprotein fusions are confirmed as described above.

EXAMPLE 2

Infection of BHK Cells by HSV-1 Incorporating scFv-Glycoprotein Fusion Proteins

[0050] The ability of the viruses incorporating ScFv-glycoprotein fusion (as produced in example 1) to infect BHK cells is analysed by single-step growth experiments and compared with 1716. The tropism of these scFv-glycoprotein fusion viruses is investigated using CHO cells that constitutively express CD55. Penetration of the viruses into CHO cells is confirmed using GFP reporter gene analysis.

[0051] Results in CHO cells will identify the factors that allow antibody-mediated viral infection and recombinant viruses will be created by cloning the appropriate scFv/glycoprotein DNA into the viral genome. The genomes of these viruses can be further modified by deletion or inactivation of glycoprotein genes such as gD, gC and gB using for example, homologous recombination.

[0052] By accumulating monoclonal antibodies to tumour specific antigens such as CEA or CD20, it is then possible to construct a panel of oncolytic herpesviruses with improved targeting to the tumour of choice.

TABLE 1

Expression in cells and incorporation into HSV1716 of scFv/gC fusion proteins.		
fusion protein	Expression in cells	Incorporation into virus
Δ164 gC	++	-
Δ193 gC	++	++
Δ215 gC	++	++
Δ227 gC	+++	++
Δ251 gC	+++	+
Δ270 gC	-	-
Δ276 gC	+++	+++
Δ313 gC	+	+
Δ326 gC	-	-
Δ328 gC	++	+++
Δ362 gC	++	++
Δ410 gC	+++	+++
Δ424 gC	+++	+++
Δ457 gC	++	+++

Expression in cells and incorporation into virus were determined by Western blotting.

+++ = strong reactivity,

++ = intermediate,

++ = weak and - = undetected

1. An HSV complex capable of targeting a specific cell type, said complex comprising an HSV modified in the terminal portion of R_L within BamHI s (0-0.02 and 0.81-0.83 mu) so as to lack neurovirulence, and a targeting agent linked to the modified HSV, wherein said targeting agent comprises an antibody binding domain.

2. (canceled)

3. An HSV complex according to claim 1 wherein said antibody binding domain is in the form of a Single Chain Variable fragment (ScFv).

4. An HSV complex according to claim 1 wherein said targeting agent is an antibody.

5. An HSV complex according to claim 1 wherein the targeting agent is capable of specifically binding to a cell surface protein of the cell type targeted.

6. An HSV complex according to claim 5 wherein the cell surface protein is a tumour associated antigen.

7. An HSV complex according to claim 6 wherein the tumour associated antigen is CEA, Her2, CD20 or CD55.

8. An HSV complex according to claim 1 wherein the targeting agent is linked to the modified virus via a viral envelope protein.

9. An HSV complex according to claim 8 wherein the targeting agent and the viral envelope protein form a fusion protein.

10. An HSV complex according to claim 8 wherein the viral envelope protein is an HSV glycoprotein.

11. An HSV complex according to claim 1 wherein the HSV has been modified such that gene γ 34.5 is incapable of expressing a functional product.

12. An HSV complex according to claim 1 wherein the HSV is HSV-1.

13. An HSV complex according to claim 1 wherein the HSV is HSV-1 strain 17.

14. An HSV complex according to claim 1 wherein the HSV is HSV1716.

15. An HSV which is capable of targeting a specific cell type, said HSV lacking an expressible γ 34.5 gene so as to lack neurovirulence and wherein said HSV expresses a targeting agent, wherein said targeting agent comprises an antibody binding domain.

16. An HSV according to claim 15 wherein said targeting agent is expressed as a fusion protein with a viral glycoprotein.

17. An HSV according to claim 15 further comprising the additional modification of one or more viral glycoproteins.

18. An HSV according to claim 17 wherein the additional one or more viral glycoproteins are deleted.

19. An HSV according to claim 17 wherein the additional one or more viral glycoproteins are modified by incorporation of the targeting agent.

20. An HSV according to claim 17 wherein the additional glycoproteins are gD, gC and/or gB.

21. (canceled)

22. An HSV according to claim 15 wherein said antibody binding domain is in the form of a Single Chain Variable fragment (ScFv).

23. An HSV according to claim 50 wherein the cell surface protein is a tumour associated antigen.

24. An HSV according to claim 23 wherein the tumour associated antigen is CEA Her3, CD20 or CD55

25. An HSV according to claim 15 wherein the HSV is HSV-I.

26. An HSV according to claim 25 wherein the HSV is HSV-I strain 17.

27. An HSV according to claim 26 wherein the HSV is HSV1716.

28. (canceled)

29. (canceled)

30. A method of producing an HSV according to claim 15 comprising:

(a) modifying the HSV genome so as to prevent γ 34.5 gene from expressing a functional protein;

(b) modifying the HSV genome by incorporating nucleic acid encoding a targeting agent so that said targeting agent is expressed as a fusion protein with a viral coat

protein, wherein said targeting agent comprises an antibody binding domain;

(c) expressing said modified HSV in a cell.

31. (canceled)

32. A method according to claim 30 wherein said antibody binding domain is in the form of a Single Chain Variable fragment (ScFv).

33. A method according to claim 30 wherein said targeting agent is an antibody.

34. A method according to claim 30 wherein the targeting agent is capable of specifically binding to a cell surface protein of the cell type targeted.

35. A method according to claim 34 wherein the cell surface protein is a tumour associated antigen.

36. A method according to claim 35 wherein the tumour associated antigen is CEA, Her2, CD20 or CD55.

37. A method according to claim 30 wherein the targeting agent is linked to the modified virus via a viral envelope protein.

38. A method according to claim 37 wherein the targeting agent and the viral envelope protein form a fusion protein.

39. A method according to claim 37 wherein the viral envelope protein is an HSV glycoprotein.

40. A method according to claim 30 wherein the HSV is HSV-I.

41. A method according to claim 30 wherein the HSV is HSV-I strain 17.

42. A method according to claim 30 wherein the HSV is HSV1716.

43-47. (canceled)

48. A pharmaceutical composition comprising an HSV complex according to claim 1 and a pharmaceutically acceptable carrier.

49. A pharmaceutical composition comprising an HSV complex according to claim 15 and a pharmaceutically acceptable carrier.

50. An HSV according to claim 15 wherein the targeting agent is capable of specifically binding to a cell surface protein of the cell type targeted.

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